Morphology, development and plant architecture of *M. truncatula*

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As many legume species, one of the specificities of *M. truncatula* is the indeterminate pattern of its development: the plant can simultaneously produce vegetative and reproductive organs. As a consequence, organs of different nature (leaves, flowers, pods) and with different physiological ages can contemporarily develop on a given plant.

1. Vegetative development

General plant morphology and architecture

A *M. truncatula* plant is made up of (1) a main axis that can organize either in a rosette (i.e. the leaves are at the level of the neck with very short internodes) or as an elongated axis; and (2) branches of different orders (e.g. primary, secondary, tertiary, branches). Plant morphology and architecture strongly vary between genotypes and are very dependent upon environmental and cultural conditions (Aitken 1955). For instance, when A17 plants are cultivated with a high plant density or with low radiations, the main axis is elongated and only very few branches are formed (erect plant). By contrast, when they are grown with a low plant density, the main axis organizes in a rosette and numerous branches develop (prostrate plant).

Each axis on a plant is composed of phytomers. A phytomer is the functional elementary unit of an axis. For *M. truncatula*, as for pea (Lecoeur 2005), a phytomer is made up of:

- An internode (section of stem between two adjacent nodes): as mentioned above, the length of the internodes can vary according to environmental conditions and according to axes. For *M. truncatula*, internodes are angular;
- A leaf: except for the first true leaf that is unifoliate, *M. truncatula* leaves are trifoliate. Each leaf is composed of (1) a petiole, (2) three leaflets which are dentate in their superior part and hairy, sometimes with a dark spot in the centre, and (3) and two basal stipules (outgrowths borne on either side of the base of a petiole) whose role is to protect the axillary bud;
- An axillary meristem, likely to produce branches (if the phytomer bearing the axillary meristem has been initiated before flower initiation) or reproductive organs (if the phytomer bearing the axillary meristem has been initiated after flower initiation).

During plant development, the phytomers on a given axis are successively initiated from the apical meristem. So, each axis is made up of a stack of phytomers whose age is different, the oldest phytomers being at the base and the youngest phytomers at the top of the axis.

Precise botanical descriptions of *M. truncatula* plants can be found in Lesins & Lesins (1979) and Prosperi et al. (1995).

Establishment of the vegetative organs

The establishment of the vegetative organs has been observed for the line A17 of Jemalong (Moreau et al., 2006), allowing identifying the main characteristics of the general organization of *M. truncatula* plant development.

The germination of *M. truncatula* is epigeal (cotyledons expand above the ground). Once the cotyledons are emitted, the leaves which are produced generally organize in a rosette which is the main axis of the plant. The phyllotaxy of the main axis for the first leaves is described in Figure 1a. The leaves which are then emitted organise with a distichous phyllotaxy (two successive leaves are placed alternatively on one side and on the other side of the axis).

During plant development, primary branches develop at the axil of the main axis leaves. The first primary branch appears at the axil of the first developed main axis leaf (the unifoliate leaf) (Figure 1a). Then the other primary branches successively appear, in the order of appearance of the main axis leaf at the axil of which they develop. Since each primary branch develops at the axil of a main axis leaf, the arrangement of the primary branches on the plant is close to the phyllotaxy of the main axis (Figure 1a). Secondary and tertiary branches generally develop at the axil of the leaves of the primary and secondary branches respectively (Figure 2). Cotyledonary branches can also be formed at the axil of the cotyledons. Branches have a distichous phyllotaxy but, due to torsions of the axes, two successive leaves can be positioned on the same side.

On a given branch, the rate of leaf appearance was shown to be constant (cf. Chapter "Mutant screening / phenology key").

A standard phenology key for characterising *Medicago truncatula* phenotypes

The phenology key has been established using the line A17 of Jemalong. However it can be used for analysing any genotype. For more details, see Moreau et al. (2006).

<u>A standard terminology for identifying organs</u>

The rosette habit of the plant makes difficult the identification of the different organs (Figure 1a). However, unambiguously identifying organs on a plant is essential as:

(1) gene expression can vary according to the position of the analyzed on the plant; so functional genomics data arising from different experiments can be compared only if they have been collected on the same organs on the plant;

(2) developmental mutants can be altered in the establishment of specific organs.

In the terminology, the main axis is called MA. A detailed analysis of the phyllotaxy of MA revealed a constant arrangement of the leaves (Figure 1a). The first true leaf that develops (the round leaf) is called leaf 0 and the trifoliate leaves are numbered in the order of their appearance, from leaf 1. In the case of developmental aberrations with a greater number of nodes with unifoliate leaves, it is the last developed unifoliate leaf which is called leaf 0, the preceding unifoliate leaves being *a posteriori* numbered with a negative notation.

Branches are named according to their position on the plant. The two cotyledonary branches are called CB1 and CB2, CB1 being the longest branch. Primary branches are called Bi, with i the rank of the main axis leaf at the axil of which they develop (Figure 1a). The terminology used for identifying secondary and tertiary branches is presented in Figure 2. Secondary branches are called Bi-j: they are identified both by the identifier of the primary branch that bears them (Bi) and by the rank of the leaf of the primary branch at the axil of which they develop (j, starting at 1). Similarly, tertiary branches are called Bi-j-k: they are identified both by the identifier of the secondary branch that bears them (Bi-j) and by the rank of the leaf of the secondary branch at the axil of which they develop (k, starting at 1).

A standard system for the notation of the plant developmental stages

Using a standard method for unequivocally describing plant developmental stages is essential:

(1) because gene expression can vary according to plant developmental stages; so functional genomics data arising from different experiments can be compared only if they have been collected at a precise developmental stage;

(2) for describing experimental conditions: for instance, the developmental stage when a treatment was applied to the plant;

(3) for characterizing mutants with developmental aberrations.

Identification of the axes on the plant

A key step for characterizing plant developmental stages consists in identifying the axes on the plant. The primary branch B0 develops at the axil of the unifoliate leaf. Consequently, having identified the unifoliate leaf and knowing the theoretical disposition of the primary branches on a plant (Figure 1a), all the primary branches can be identified. The secondary and tertiary branches are identified both by the rank of the axis that bears them and by the number of the leaf at the axil of which they develop (Figure 2). When two or more primary branches develop at the axis of a given main axis leaf, they can be differentiated because the length of Bi is superior to that of Bi', the length of Bi' is superior to Bi", and so on. This rule is valid for the secondary, tertiary and cotyledonary branches.

In order to identify more easily and more quickly the primary branches throughout plant cycle, it is recommended to label them with pencil marks or small clips as they appear.

System of phenological notation

In reference to other systems of notation, our system separates descriptions for vegetative and reproductive development, in order to accommodate the many vegetative-reproductive development relationships that can occur in indeterminate plants.

Vegetative developmental stages are determined by counting the number of appeared trifoliate leaves per axis. Notations can be performed on all the axes of the plant or on a smaller number of selected axes. In the system of notation, a leaf is counted when it is completely unfolded, and a leaf is considered as completely unfolded when its central leaflet is planar (Figure 3e). In addition, in order to accommodate intermediate stages occurring between the unfolding of two successive leaves on an axis, the system of notation includes a decimal code. For each axis, the decimal code applies to the last leaf that is not completely unfolded. It consists of three intermediate stages (0.25, 0.50 and 0.75) corresponding to the degree of unfolding of the central leaflet of the last leaf (Figure 3b, c and d respectively).

Reproductive developmental stages are determined (1) by identifying for each axis the position of the first reproductive node and (2) by counting the number of reproductive nodes per axis. Notations can be performed on all the axes of the plant or on a smaller number of selected axes. A node is considered as reproductive from the moment when it bears at least one open flower (Figure 3f).

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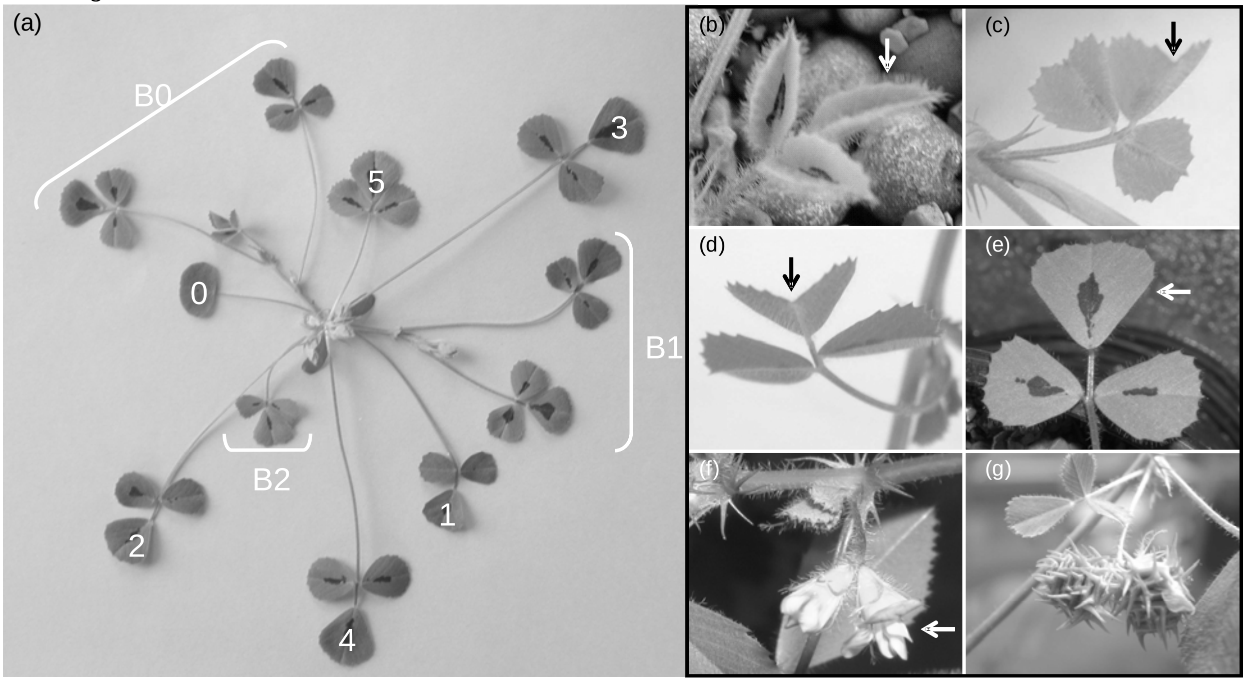

Figure 1

Arrangement of the vegetative organs on the main axis of *Medicago truncatula* A17 and decimal code used for the notations of the development
From Moreau et al. (2006)

(a) Phyllotaxy of the main axis and arrangement of the primary branches
Main axis leaves are numbered according to their rank of appearance from 0 (i.e. the unifoliate leaf) to 5. Leaves can be produced in the clockwise turn or not. From the leaf 6, leaves generally organise with a distichous phyllotaxy. The primary branches (B0, B1 and B2) are named according to the rank of the main axis leaf at the axil of which they develop

(b) to (g) Decimal code used for the notation of the vegetative development, and definition of the reproductive developmental stages for *Medicago truncatula*.
(b) The decimal code is 0.25: the three leaflets are separated from each other, and the edges of the central leaflet are separated with an acute angle.
(c) The decimal code is 0.50: the edges of the central leaflet form a right angle.
(d) The decimal code is 0.75: the edges of the central leaflet form an obtuse angle.
(e) The decimal code is 0.00: the limb of the central leaflet is planar.
(f) In the system of phenological notation of the reproductive developmental stages, a node is considered as reproductive from the moment when it bears at least one open flower as indicated by the arrow.
(g) Pods on a reproductive node.

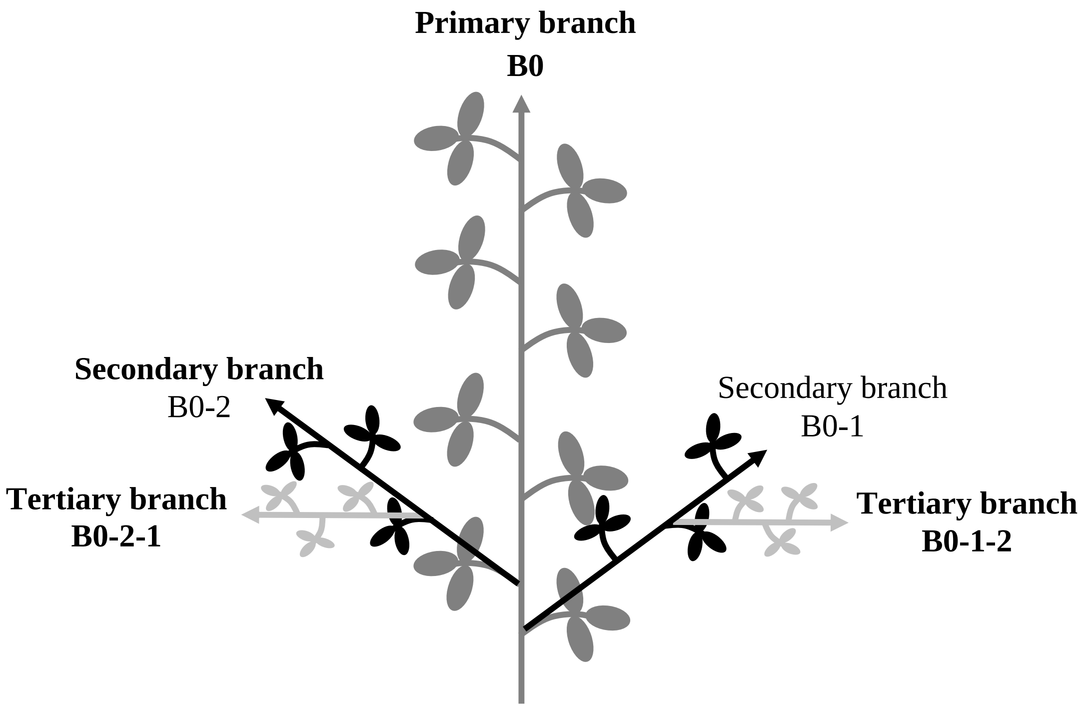

Figure 2
Diagram of the arrangement of the secondary and tertiary branches on a primary branch of *Medicago truncatula*.
From Moreau et al. 2006

The example of the primary branch B0 is taken. Each arrow represents a branch. For each branch, the leaves are numbered according to their rank of appearance, from the base to the apex. Secondary and tertiary branches are identified both by the identifier of the branch that bears them and by the rank of the leaf at the axil of which they develop.